

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. – 10. (cancelled)

11. (currently amended) A method of evaluating the deacetylation of a substrate in the presence of a Sir2 protein, NAD or an NAD-like compound and an agent, testing an agent for ability to alter deacetylase activity of a SIR2 protein the method comprising:

a) combining a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced Sir2 protein, NAD or an NAD-like compound and an agent to be tested, thereby producing a combination; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

12. – 168. (canceled)

169. (new) The method of claim 11 wherein the determining comprises electron-spray mass spectroscopy.

170. (new) The method of claim 11 further comprising comparing deacetylation of the substrate in the presence of the agent to deacetylation of the substrate in the absence of the agent, wherein a difference in substrate deacetylation indicates that the agent alters Sir2 protein deacetylase activity.

171. (new) The method of claim 11 wherein the Sir2 protein is a human Sir2 protein.
172. (new) The method of claim 11 wherein the Sir2 protein is a murine Sir2 protein.
173. (new) The method of claim 11 wherein the Sir2 protein is a fusion protein.
174. (new) The method of claim 11 wherein the substrate is a fragment of a histone that comprises the N-terminal tail of a histone protein.
175. (new) The method of claim 174 wherein the histone protein is histone H3.
176. (new) The method of claim 175 wherein the fragment is acetylated at positions corresponding to the lysine amino acid residue is lysine 9 and/or lysine 14 of H3 histone.
177. (new) The method of claim 11 wherein the substrate is a histone protein.
178. (new) The method of claim 177 wherein the histone protein is selected from the group consisting of an H2B, H3 and H4 histone protein.
179. (new) The method of claim 177 wherein the histone protein is acetylated on a lysine amino acid residue.
180. (new) The method of claim 179 wherein the histone protein is histone H4 and the protein is acetylated on lysine 16 of histone H4.
181. (new) The method of claim 11 wherein the acetylated amino acid side is a lysine.

182. (new) The method of claim 11 wherein the Sir2 protein is an isolated Sir2 protein.

183. (new) The method of claim 11 wherein the Sir2 protein is a recombinantly produced Sir2 protein.

184. (new) The method of claim 11 wherein the combination comprises  $\text{MgCl}_2$ .

185. (new) The method of claim 11 wherein the combination comprises DTT.

186. (new) The method of claim 11 further comprising formulating the agent with a pharmaceutically acceptable carrier to provide a pharmaceutical composition.

187. (new) The method of claim 186 wherein the pharmaceutically acceptable carrier comprises a carbohydrate.

188. (new) The method of claim 11 wherein the combination comprises NAD.

189. (new) The method of claim 11 wherein the Sir2 protein is a Sir2 $\alpha$  protein.

190. (new) The method of claim 189 wherein the Sir2 $\alpha$  protein comprises SEQ ID NO:12.

191. (new) A method of evaluating the deacetylation of a substrate in the presence of a human Sir2 protein, NAD, and an agent, the method comprising:

a) providing a mixture comprising a substrate that comprises an acetylated amino acid side chain, an isolated or recombinantly produced human Sir2 protein, NAD, and an agent to be tested; and

b) determining if the acetylated amino acid side chain in the substrate is deacetylated.

192. (new) The method of claim 191 wherein the mixture comprises  $MgCl_2$ .

193. (new) The method of claim 191 wherein the mixture comprises DTT.

194. (new) The method of claim 11 or 191 wherein the SIR2 protein is produced in *E. coli*.

195. (new) The method of claim 11 or 191 wherein the agent is a protein.

196. (new) The method of claim 11 or 191 wherein the agent is a peptide.

197. (new) The method of claim 11 or 191 wherein the agent is naturally occurring.

198. (new) The method of claim 11 or 191 wherein the agent is non-naturally occurring.

199. (new) The method of claim 11 or 191 wherein the agent is chemically synthesized.

200. (new) The method of claim 11 or 191 wherein the agent is a carbohydrate.

201. (new) The method of claim 11 or 191 wherein the agent is a steroid.
202. (new) The method of claim 11 or 191 wherein the agent is a lipid.
203. (new) The method of claim 11 or 191 wherein the agent is an anion.
204. (new) The method of claim 11 or 191 wherein the agent is a cation.
205. (new) The method of claim 11 or 191 wherein the agent is an oligonucleotide.
206. (new) The method of claim 195 wherein the agent is an antibody.
207. (new) The method of claim 191 wherein the Sir2 protein is an isolated Sir2 protein.
208. (new) The method of claim 191 wherein the Sir2 protein is a recombinantly produced Sir2 protein.
209. (new) A method of evaluating deacetylation of a substrate in the presence of a Sir2 core domain, and NAD, the method comprising:
  - a) providing a mixture comprising a substrate that comprises an acetylated lysine amino acid side chain, a recombinantly produced protein that comprises a SIR2 core domain, and NAD; and
  - b) determining if the acetylated amino acid side chain in the substrate is deacetylated.
210. (new) The method of claim 209 wherein the recombinantly produced protein comprises a human SIR2 core domain.

211. (new) The method of claim 209 wherein the mixture comprises  $\text{MgCl}_2$ .

212. (new) The method of claim 209 wherein the mixture comprises DTT.

213. (new) The method of claim 209 wherein the recombinantly produced protein is a fusion protein.

214. (new) The method of claim 209 wherein the recombinantly produced protein is produced in *E. coli*.